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MICROBIAL LOAD MONITOR



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MCDONNELL DOUGLAS ASTRONAUTICS COMPANY - ST. LOUIS

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MICROBIAL LOAD MONITOR

1 NOVEMBER 1977

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1.0 SUMMARY

Work under Contract NAS 9-11877 to provide a total design of a Microbial Load Monitor (MLM) system flight engineering model, continues as scheduled. Activities during this contractual quarter include fabrication and checkout of the Card taper and the media pump system, fabrication of the final two incubating reading heads, Sample Receiving and Card Loading Device (SRCLD) assembly and related sterility testing, and Software. Progress in these areas is summarized below.

1.1 Fabrication of the Card Taper is complete and taped Cards look good.

1.2 The Media Pumping System has been fabricated and is undergoing checkout and calibration. Initial tests of the system show repeatable results. Cross contamination was improved by a redesign of the dispensing head before fabrication.

1.3 SRCLD assembly is a continuing item for the contract.

1.4 Two additional incubating reading heads have been machined, assembled and checked out. A design modification in the detector electronics has eliminated a source of reading instability.

1.5 SRCLDs were tested for sterility. An electron beam sterilizer was used at two different levels. A level of 2 millirads will be used in production. All packaged SRCLDs will be sterilized.

1.6 Software is progressing in two areas - the supervisory program (with five operating heads) and the plot program. Hardware stack limitations have forced a programming technique change by using random access memory for a software stack.

2.0 TECHNICAL DISCUSSION

Major items for this reporting period are Card Taper, Media Pumping System, Sample Receiving and Card Loading Device assembly, Incubating Reading Heads, Sterility Testing, and MLM software.

2.1 Card Taper - During this quarter the MLM Card taper design was finalized, the unit was fabricated, and successfully tested. Figure 1 is a photograph of this unit.

The design of the taper had to be modified because of a change in the tape. The original machine was designed to use 3M manufactured tape that did not have a liner. Due to problems with this tape and the reluctance of the manufacturer to continue to market this product, it was decided to find another source. We now procure this tape from Fasson. It is chemically the same as the 3M tape with the same adhesive but comes with a release liner. This eliminated the problem of tape sticking to itself and made it easier to apply. However, the taper had to be redesigned to add a take-up reel for the liner. Other than the addition of this take-up reel, the taper is basically the same as the original design. The design was finalized after this addition. Figure 2 shows the take-up reel on top and the slip clutch necessary to achieve liner tension.

Fabrication, assay, and test of the unit went very smoothly. There were no modifications required due to fabrication or test problems. The unit came up working.

Operation of the taper is very simple. The Cards are loaded into the hopper and feed one at a time by rotating the handle from right to left about 145° (Figure 1). A Card drops onto the track and is pushed into the application rollers by rotating the handle back left to right. This continuous action pushes one Card against the next through the application roller and then through the buffer rollers. The Cards come out butted together on one continuous strip of tape and are separated by cutting them apart with an exacto knife. The action of the Card going through the application rollers pulls the tape from its roll, removes the liner, and winds the liner onto the liner take-up reel.

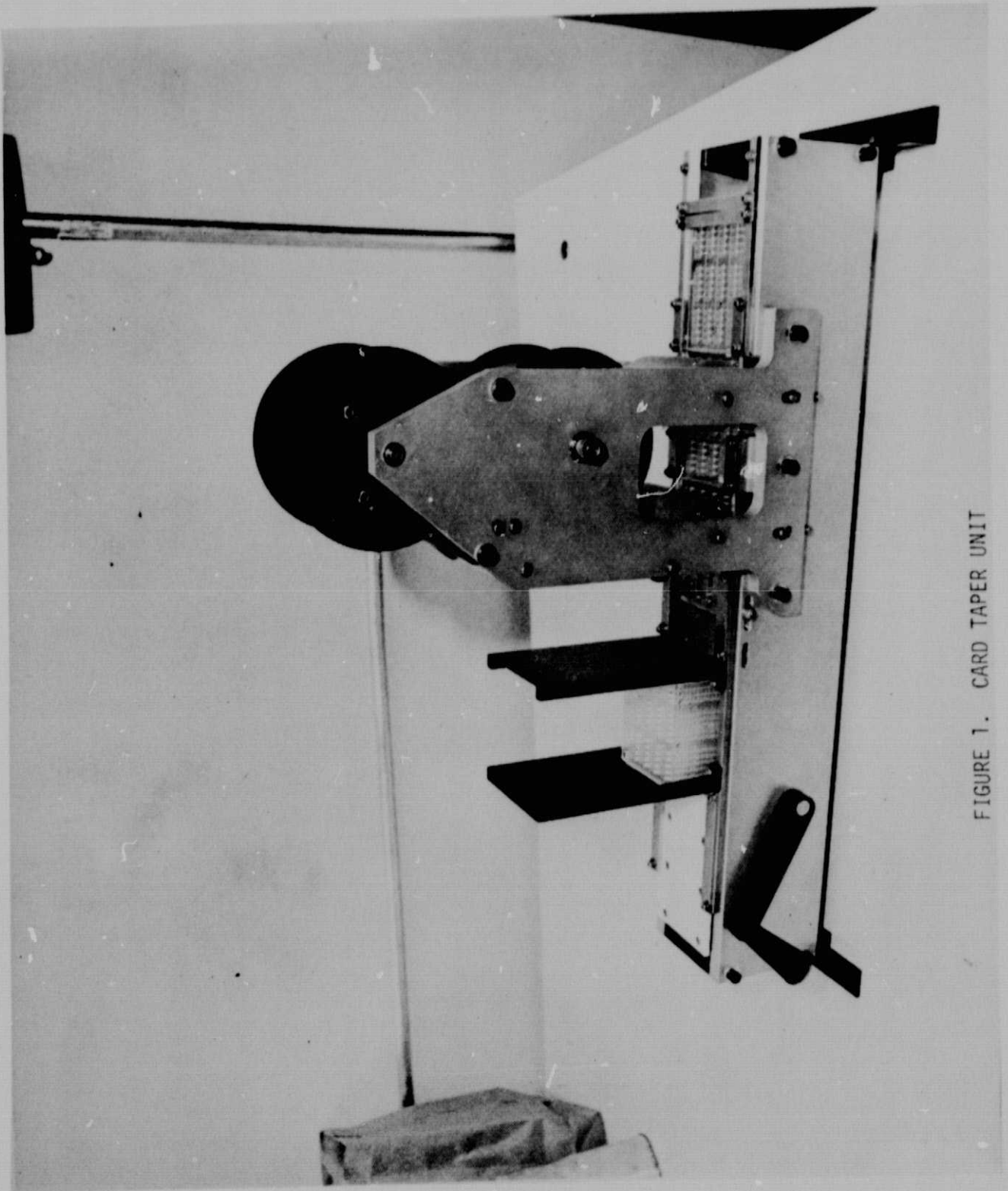


FIGURE 1. CARD TAPER UNIT

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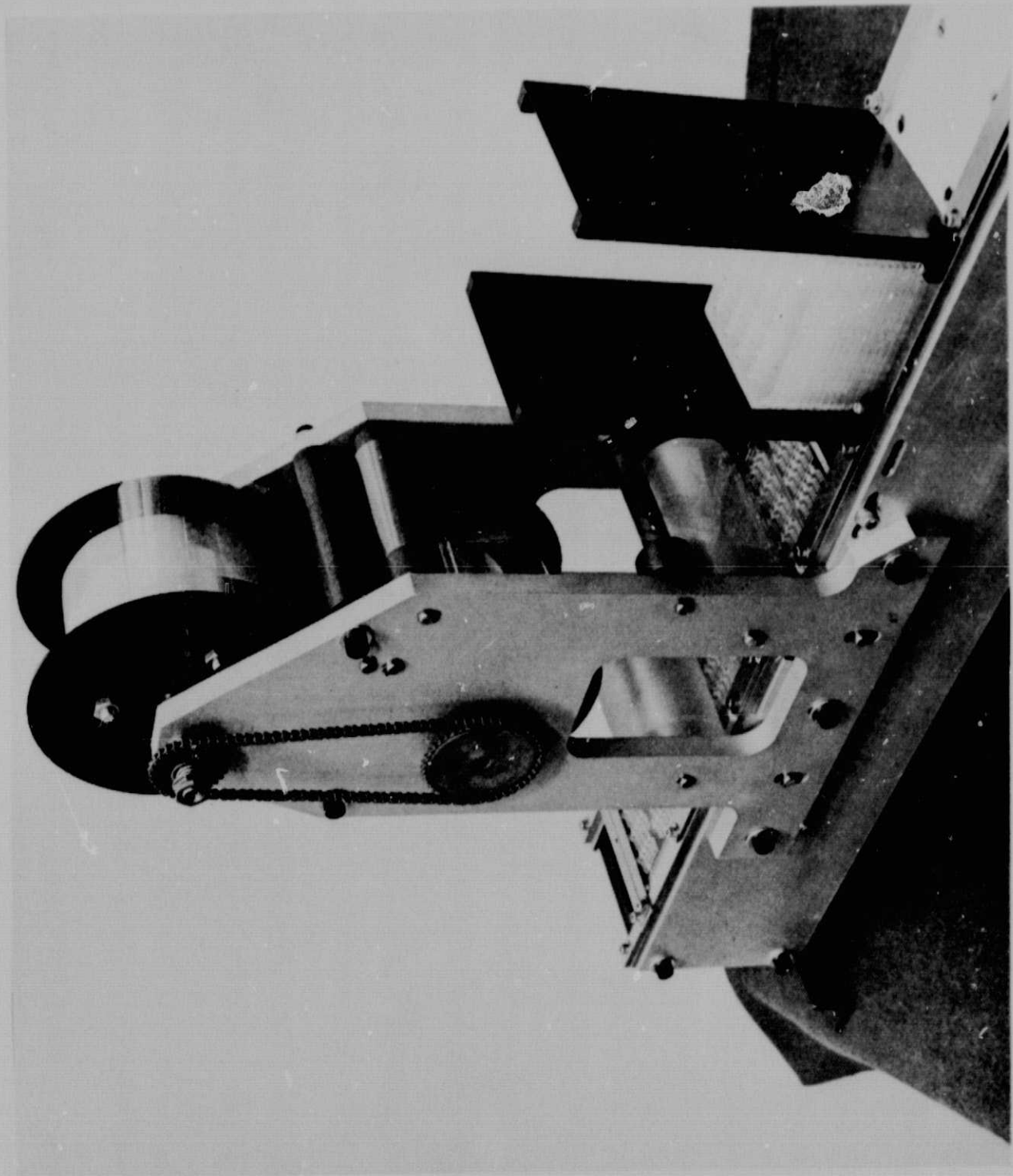


FIGURE 2. CARD TAPER UNIT (REAR VIEW)

2.2 Media Pumping System - The media pumping system as shown in Figure 3 is comprised of two units, the media pumping station (Figure 4) and the dispensing station (Figure 5). This pumping system can dispense up to 60 channels simultaneously.

Each pumping channel contains a Hamilton Microliter syringe, a 20 gauge needle attached to the syringe, tubing, and a 20 gauge dispensing needle. The pumping station also contains the motor, cams, and switches to drive the pump as seen in Figure 4. The dispensing station (Figure 5) contains the dispensing head, the media reservoir, a tray with handle for holding the Cards, a manual crank for raising and lowering the dispensing head, and control switches.

The design of this system was finalized early in this quarter after changing the design of the dispensing station. The original design called for the dispensing head to translate from the media holder to the Card horizontally. There was some concern that during this translation media of one type may accidentally drop off the dispensing needle into another media reservoir, thus contaminating it. This translation action was eliminated and replaced by movement of the Card and associated tray.

The fabrication, assembly, and initial checkout of these units went very smoothly. No redesign or modifications were required to make it operate properly. Calibration of each pumping channel is presently in progress. Each channel is calibrated gravimetrically for the desired amount of media. This ranges from 18 to 20 microliters with a tolerance of $\pm 2\%$. Calibration is accomplished by dispensing 10 drops of distilled water, weighing them on a scale and adjusting the bolt at the top of the pumping unit until the desired results are obtained. All plungers are pulled to the same height and the bolt determines the low end of the stroke.

To operate this system, each pumping channel must first be filled with proper media so that there is no air in the system. This is done by first elevating the dispensing station above the syringes so that media will flow from the dispensing needle to the syringe. With the needle at the end of the syringe disconnected from the syringe and the plunger completely depressed, media is allowed to flow until it flows out of the syringe needle. The needle is then connected to the

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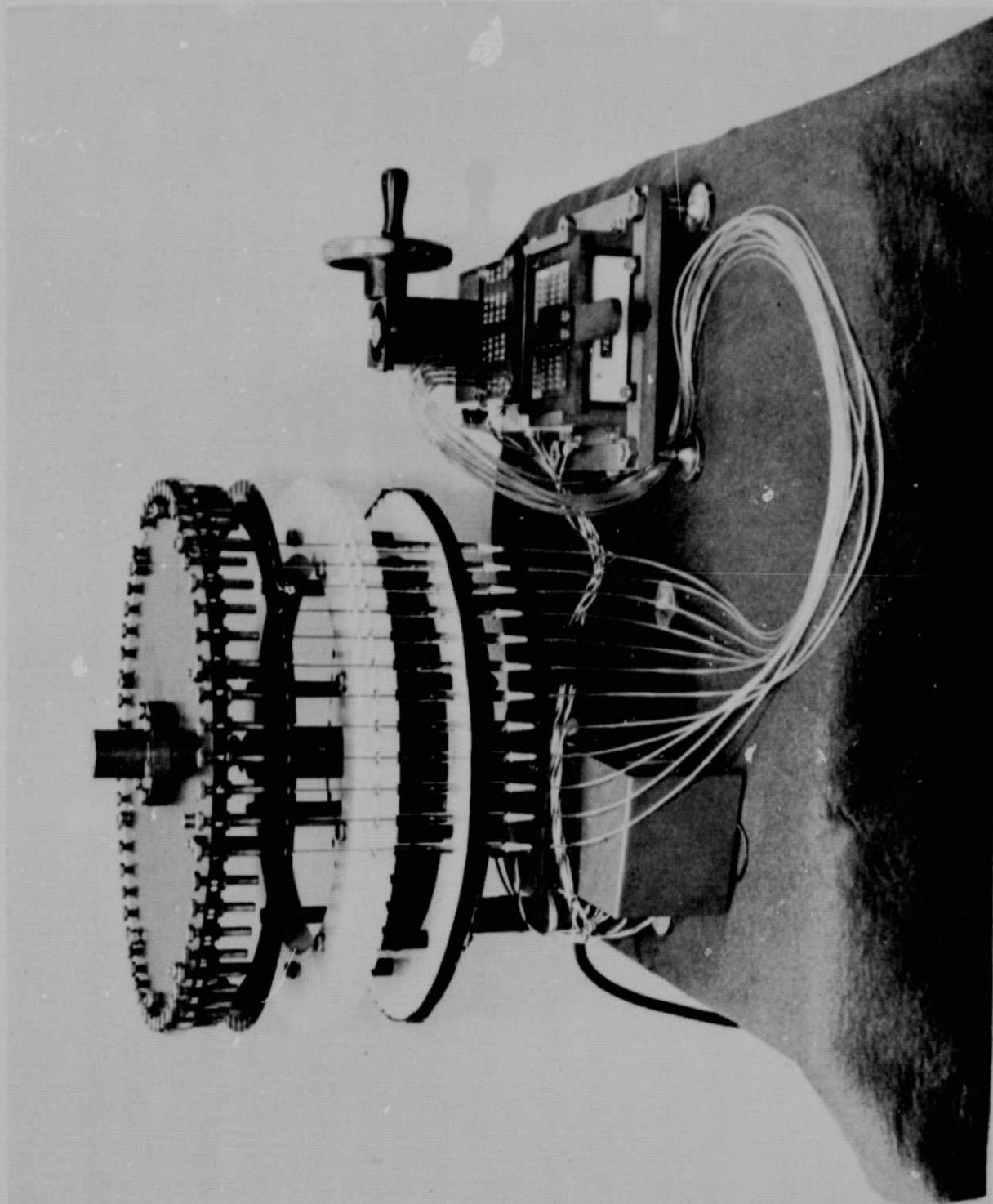


FIGURE 3. MEDIA PUMPING SYSTEM

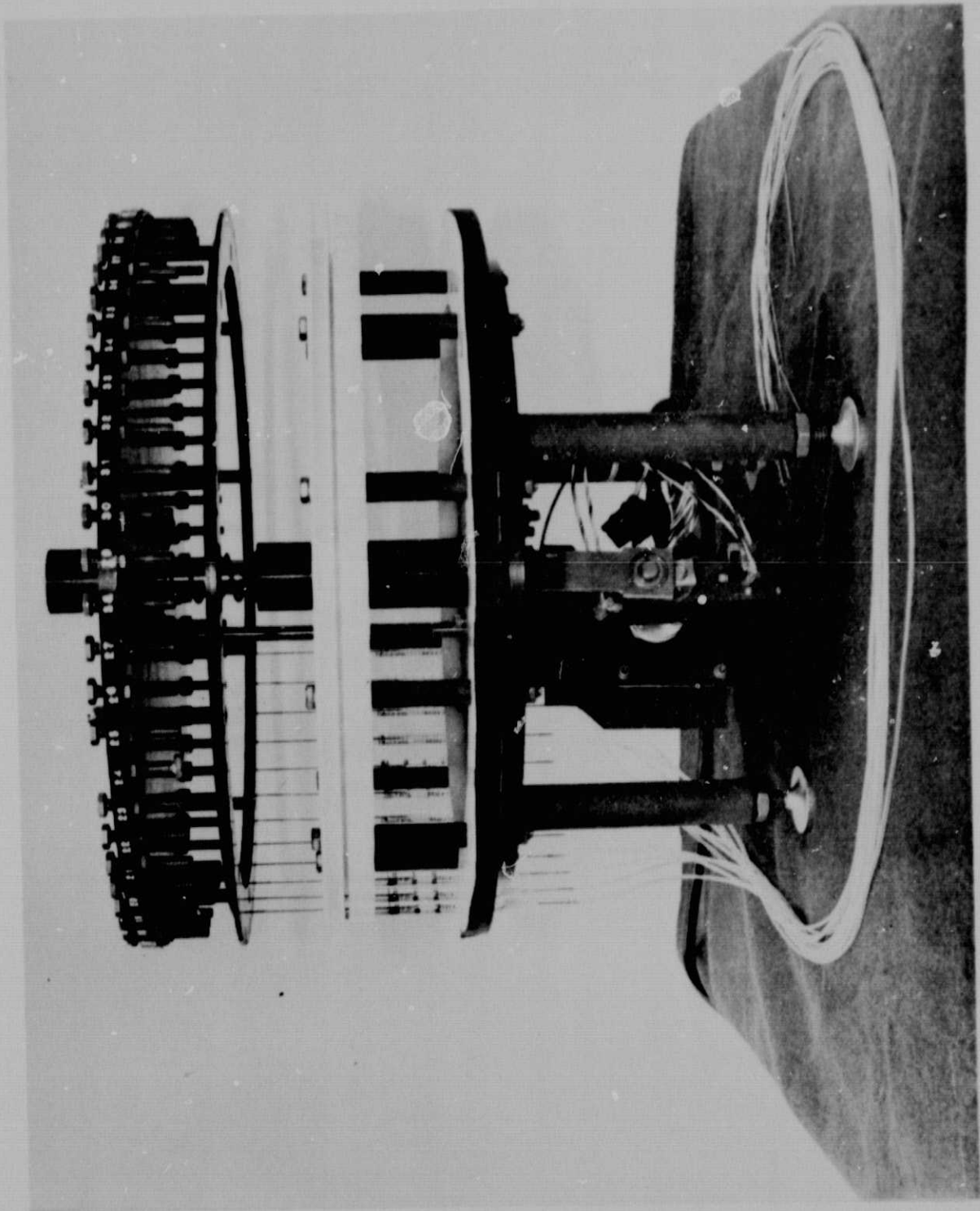


FIGURE 4. MEDIA PUMPING STATION

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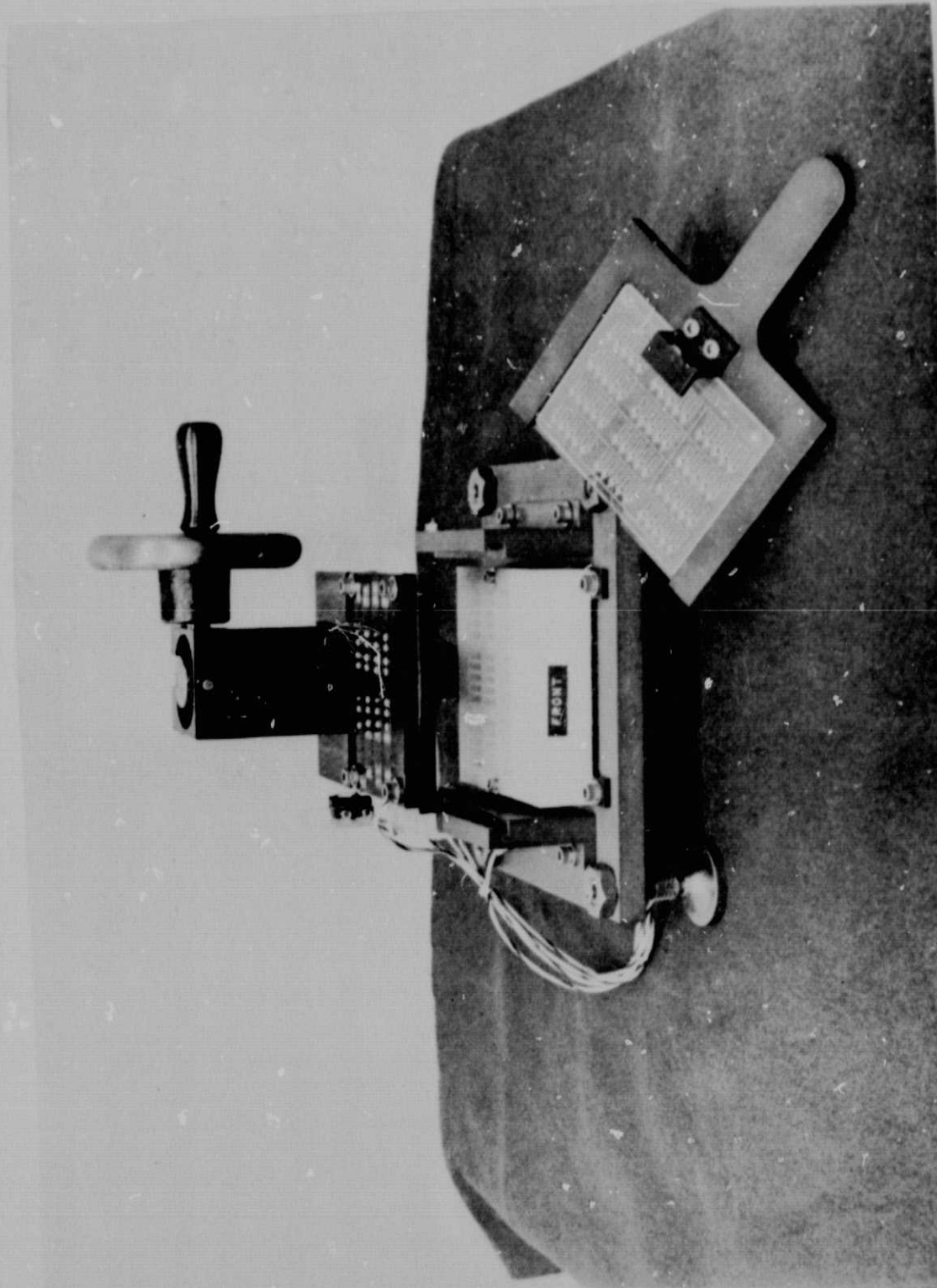


FIGURE 5. MEDIA DISPENSING STATION

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syringe, and plunger completely pulled out, and when all the air is displaced by the media the plunger is reinserted into the syringe. This is done for all the channels required for the particular Card to be filled. When this purging operation is completed, the dispensing station can be lowered to its normal height and the filling of Cards can start.

A Card is filled by first placing it onto the Card tray. The Card tray has two dowel pins which fit in the Card alignment hole to aid in lining up the Card with the dispenser and prevents the Card from being put into the tray in the wrong orientation. Before inserting the loaded tray into the dispensing station, the dispensing head is lowered until a mechanical stop is reached. This inserts the dispensing needles into the media and activates a micro switch which energizes the pump motor. All the syringe plungers are pulled up sucking the media into the needles. The head is then hand cranked to its uppermost position, the Card tray inserted and the dispensing head lowered until it hits the stop on the tray. The tray insertion activates one switch and the head another. This combination energizes the pump motor again and the syringe plungers are pushed down dispensing the proper amount of media into each hole. This action is repeated until the desired number of Cards are filled. One media reservoir can fill approximately fifty cards. Additional reservoirs are used for additional Cards.

2.3 Sample Receiving and Card Loading Device Fabrication - Fabrication of Sample Receiving and Card Loading Devices (SRCLDs) is an ongoing item. Devices are septumized, cured, and assembled in batches by a technician as time allows. The bottom piece is now ultrasonically welded instead of gluing with plastic solvent. Only if testing reveals pinhole leaks is solvent used and then only on the immediate area of the leak.

Ultrasonic welding to date has produced nearly leak-free units after the initial welds are made to calibrate the machine for the SRCLD. After assembling and leak-checking the SRCLDs are packaged and sterilized with an electron beam sterilizer.

2.4 Reading Head Fabrication and Checkout - Two complete incubating reading heads were fabricated and checked out this reporting period. One improvement has been made in the detector electronics. Spare arrays are still to be fabricated.

Mastercraft Tool Company, the machining company for the previous heads, was also selected for the final two heads. After checking for fit, all outside parts were clear anodized for surface protection. Each head was then assembled with all electronics, heaters, and arrays.

Electrical checkout of one assembled head revealed a sawtooth oscillation on the reference voltage. A possible array problem proved negative when the detector arrays were removed and the oscillation remained. Subsequent investigation revealed the Analog Devices AD580 voltage reference would oscillate with a capacitive load of from 0.68 μ f to 2 μ f. We were using 1 μ f for transient filtering. A telephone conversation with the manufacturers application engineer revealed that the device is capacitive load sensitive but he was surprised that 1 μ f would cause the oscillation. All incubating reading heads have now had this capacitor removed even though oscillations were not seen on all heads and the remaining checkout proceeded smoothly. Subsequent tests on other heads revealed more stable values on some heads with the capacitor removed even though the sawtooth oscillation did not seem to be present.

2.5 Sterility Testing - Sterility tests were conducted on assembled Sample Receiving and Card Loading Devices (SRCLDs). Four units in each of the following three groups were used. Electron beam sterilization was employed.

Group A: No sterilization

Group B: 2 millirads sterilization

Group C: 3 millirads sterilization.

Groups B and C had no growth and Group A had growth which was less than 50 c.f.u./ml. Although this is not considered a significantly high count, these numbers would give positive results with enumeration media and thereby alter enumeration results.

Physical effects of the electron beam sterilization on the SRCLD seem limited to an immediate color change in the plastic (from clear to yellowish) which is not permanent and gradually fades. Based on the above results, electron beam sterilization will be employed on assembled and packaged units.

2.6 Software - Software is progressing in two areas. One is the MLM supervisory program and the other is the rough plot program. Due to the length of the

programs, the plot program overlays a portion of the data gathering's storage area. A change in programming techniques is also underway.

2.6.1 Microbial Load Monitor Supervisory Program - When all routines are included, this program will be able to handle all aspects of MLM operation except plots which will be handled by a separate program. It is now able to monitor five incubating reading heads and store the resulting data on magnetic tape in a one record per reading per head format. Sixteen hours of half-hour readings for five heads results in 160 different data records and five calibration type records.

Routines for calculating percentage change for a channel and relating it to a media or antibiotic abbreviation will be extracted from the plot program. Operator commands for controlling the supervisory program will be added in the coming months.

2.6.2 MLM Plot Program - Time history profiles for each channel are plotted after the five heads have all been inactivated (i.e., no active Cards remain in the head). The space needed for plot data and formats precludes simultaneous data gathering and plotting.

Time history profiles are plotted to a four percent accuracy every half-hour. This is a compromise between plotting accuracy and plotting time. Headings list incubating head number, channel number associated with each plot symbol, and the related media or antibiotic abbreviations. Table 1 shows the position of each operator channel number and its related abbreviation. These are different than those used by the hardware. A table look-up is used to match operator channel with hardware channel. Henceforth, when channel is mentioned it will mean the operator related channel.

Table 2 relates the one or two character abbreviation used in the MLM and its plots to the media and antibiotics used in the Card. These abbreviations, based on industry usage, have been selected to prevent duplication and to make maximum use of MLM memory space and limited 16 character alphanumeric display.

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TABLE 1
CHANNELS AND ABBREVIATIONS

1 EC	2 <u>FD</u>	3 SX	4 NA	5 AM	21 PS	22 <u>AN</u>	23 NN	24 GM	25 CB	41 AH	42 <u>K</u>	43 GM	44 NA	45 TE
6 KE	7 <u>GM</u>	8 SX	9 NA	10 TE	26 SR	27 <u>GM</u>	28 SX	29 NA	30 K	46 GA	47 BT	48 <u>AM</u>	49 P	50 ER
11 CI	12 <u>FD</u>	13 SX	14 NA	15 TE	31 GD	32 <u>FD</u>	33 ER	34 TE	35 <u>AM</u>	51 YE	52	53	54	55 +C
16 PR	17 <u>K</u>	18 SX	19 NA	20 AM	36 SA	37 <u>CF</u>	38 ER	39 TE	40 <u>CC</u>	56 EN	57 EN	58 EN	59 EN	60 EN

NOTE: Underlined antibiotics are associated with the media directly to their left.

TABLE 2
ABBREVIATIONS AND NAMES

<u>Antibiotics</u>	<u>Media</u>
FD = Nitrofurantoin	EC = E. Coli
SX = Trimethoprim-Sulfamethoxazole	KE = Klebsiella-Enterobacter
NA = Nalidixic Acid	CI = E. freundii
AM = Ampicillin	PR = Proteus
GM = Gentamicin	PS = Pseudomonas aeruginosa
K = Kanamycin	SR = Serratia
ER = Erythromycin	GD = Group D Enterococcus
CF = Cephalothin	SA = Staph aureus
P = Penicillin	AH = Acinetobacter-Herellea
TE = Tetracycline	GA = Group A Beta Strep
CC = Clindamycin	BT = Beta Strep Broth
CB = Carbenicillin	YE = Yeast
AN = Amikacin	+C = Control
NN = Tobramycin	EN = Enumeration

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2.6.3 Programming Technique Change - The IMP-16C has a hardware stack used for saving return addresses and register values which may be needed after a subroutine call. This stack is only sixteen words deep. Pushing more than sixteen words on the stack means early stored values may be lost. Due to the many depths of subroutine calls within subroutines, this has occurred within the plot program.

The new technique is to use the hardware stack only for return addresses where it must be used. All register saves will be done with software in a random access memory stack initialized at power-up. Writing of the basic routines and modification of the plot program is now underway.

3.0 PROGRAM STATUS

The MLM System Engineering model reached the following levels at the end of this contractual quarter. Five incubating reading heads are operating. The Card Taper and Media Pumping System are also fabricated and checked out. Software is progressing well. Microbiological testing is ready to begin and will be the major item during the next reporting period.